

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA*

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ABSTRACT

The aim of the present study was to find out antibacterial property of *Moringa oleifera*, family Moringaceae. *Moringa oleifera* is a very useful tree in tropical countries. In ayurvedic all parts of the tree used in different healing procedures for different diseases. The plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus epidermidis* bacteria were used. Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against *Streptococcus mutans* and aqueous extract shows maximum activity against *Proteus vulgaris*.

KEYWORDS: Antibacterial Activity, *Moringa oleifera*, Phytochemical Screening

INTRODUCTION

Moringa oleifera is one of the species of family *Moringaceae*, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the Pacific and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mothers best friend” (Julia coppin, 2008). *Moringa oleifera* is commonly known as “Drumstick”. It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract (Trupti Rastogi et al., 2009). *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark (Roloff A, 2009). The *Moringa oleifera* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals (Pal K et al, 1995).

The leaves are outstanding as a source of vitamins A when raw as a source of vitamin C. They are also good sources of vitamin B and are among the best plant sources of minerals (Talhaiani P et al., 2000). Ethanolic extract of *Moringa oleifera* leaves contain niazirin, niazirin, niazirinins A and B (S. Faiji et al., 1994). Benzoic acid, gallic acid, beta benzaldehyde have been isolated from methanolic extract of *Moringa oleifera* leaves (L.O. manguro et al, 2007). Leaves of this plant are reported to possess various biological activities, including hypocholesterolemic, antidiabetic,

hypertensive agent (K. Mehta et al., 2003; A.Kar et al., 2003; S.faiji et al., 1995; AP Guevara et al 1999), and regulate thyroid hormone (P. tahiliani et al., 2004), central nervous system, digestive system, nutrition and metabolism eye, ear nose throat genito-urinary system (KM. Nadkarni et al, 2007), to treat gastric ulcers (S. Pal et al 1995) and scurvy (D. Selvakumar et al, 2008). Reports have also described the plant to be highly potent anti-inflammatory agent (I.C. Ezeamuzle et al., 1996) and antitumour activity (A. Murakami et al, 1998). The plant has also been reported to be hepatoprotective against antitubercular drug such as isoniazid and rifampicin (L. Pari et al 2002, S. Fakurazi et al 2008). *Moringa oleifera* is also being studied for its anti-inflammatory, antimicrobial, diuretic (A. Caceres et al 1991; A. Caceres et al., 1992; S.L. Udupa et al 1994), antibiotic (U. Eilert et al 1981), hypotensive (S. Faiji et al., 1994), and antimicrobial properties (U. Palaniswamy et al., 2004).

An immune enhancing polysaccharide (S. Mondal et al, 2004) and niaziminin, having structural requirement to inhibit tumour promoter induced Epstein Barr virus activation have been reported from the leaves (A. Murakami et al, 1998). The alcoholic extract of leaves of *Moringa oleifera* were reported to have analgesic activity (G.S. Nitin et al., 2008). Traditionally, the plant is used as antispasmodic, stimulant, expectorant and diuretic (K.M. Nadkarni et al., 2009). *Moringa oleifera* is used as drug by many ayurvedic practitioners for the treatment of asthma and to evaluate the anthelmintic activity of methanolic extract of *Moringa oleifera* in adult Indian earthworms *Pheretima posthuma* at different doses (Iswar Chandra et al, 2010).

MATERIALS AND METHODS

Collection of Plant Materials

The experiment was conducted in the year 2013 in the college laboratory. Leaves were collected from the *Moringa oleifera* plant (Figure 1a,b) from the herbal garden. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.



Figure 1(a, b): *Moringa oleifera*

Preparation of Leaf Extracts

Fresh leaves (20-30 gm) of *M. oleifera* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 5 days. The dried leaves were ground into powder using a mortar and pestle. 25 g of the powdered leaves were separately extracted in 500ml conical flasks with 90% ethanol (Ethanol extraction) and water (Aqueous extraction). The conical flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional manual agitation of the flask using a sterile glass rod at every

24 hour. The extracts were separately filtered using sterile Whatman no. 1 filter paper. These extracts (Ethanollic and aqueous) were used in further process.

Phytochemical Analysis

Phytochemical analysis of extract for qualitative detection of alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extracts.

Alkaloids

- Wagner's test
- Dragandroff test
- Hager test
- Baljet test

Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken. 1ml of 10% NaOH solution was added to the mixture.

Saponins

Frothing Test: 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

Steroids

Salkowski Test: 5 drops of concentrated H₂SO₄ were added to 1ml of each extract in a separate test tube.

Tannins

2ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution were added to each extract.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added.

Reducing Sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

Volatile Oil

2ml of Extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl.

Source of Microorganisms

The organisms used were *Escherichia Coli* (MTCC No.40), *Pseudomonas aeruginosa* (MTCC No.424), *Staphylococcus aureus* (MTCC No.87), *Proteus vulgaris* (MTCC No.742), *Streptococcus mutans* (MTCC No.497), *Bacillus subtilis* (MTCC No.441) and *Staphylococcus epidermidis* (MTCC No.9041). The organisms were obtained from MTCC Chandigarh and maintain according to specification. Sub culturing was done at the interval of 15 days.

Determination of Antibacterial Activity

The antibacterial activity of the *Moringa oleifera* leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells of 6mm were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 24 hours and the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial activity of the different extracts were evaluated by comparing their zones of inhibition with standard antibiotic streptomycin.

RESULTS AND DISCUSSIONS

The present study reveals that *Moringa oleifera* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 1.

Table 1: Qualitative Phytochemical Screening of Ethanol and Aqueous Leaf Extract of *Moringa oleifera*

Solvents Used for Extraction	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Glycoside	Reducing Sugar	Volatile Oil
Ethanol	+	+	+	+	+	—	—	—
Water	+	+	+	+	—	—	—	+

Antibacterial activity of *Moringa oleifera* was seen against several bacteria namely *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus epidermidis*. The ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris* as shown in the Table 2.

Table 2: Antibacterial Activity of Ethanol and Aqueous Leaf Extract of *Moringa oleifera*

Name of Microorganism	Zone of Inhibition (mm)		Standard (Streptomycin)
	Water Extract	Ethanol Extract	
<i>Escherichia Coli</i>	01	—	6
<i>Staphylococcus aureus</i>	04	—	7
<i>Proteus vulgaris</i>	06	03	7
<i>Pseudomonas aeruginosa</i>	—	05	8
<i>Bacillus subtilis</i>	05	04	6
<i>Staphylococcus epidermidis</i>	01	03	6
<i>Streptococcus mutans</i>	03	06	7

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades, 1979). Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina et al., 1997). Plant terpenoids are used extensively for their aromatic qualities.

They play a role in traditional herbal sonedies and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions (Yamunadevi et al., 2011).

Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells (Winter et al., 1993). The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Moringa Oleifera* showed good antibacterial activity against several organisms like *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *E.coli* as supported by previous studies.

CONCLUSIONS

The present study conclusively demonstrates that *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids. The antibacterial activity *Moringa oleifera* was clearly shown by the present study against various test organisms like *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilus*, and *Staphylococcus epidermidis*. All these preliminary reports warrant an in depth analysis of the usefulness of *Moringa oleifera* as miracle drug against various ailments.

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